

Corrigendum

ad Atalanta 12: 368-385.

S. 369 15. Zeile, Mesopotamien, nec Mesopotanien
S. 370 7. Zeile, des. et corrig., nec et corrig.
S. 371 Skizze re. u., techn. Fehler, 66 % streichen
S. 373 22. Zeile, Nominatform, nec Nomonatform
S. 376 20. Zeile, Tegulae, nec Tegula
S. 378 Fig. 2, aus drucktechn. Gründen verkleinert
S. 381 Fig. 30, Photo P. STRAUSS, nec A. HOFMANN
S. 381 ad Fig. 31 (Fig. 27, 28). Der Fundort am Suvarihalil-Paß befindet
sich nicht wie von BETTI laut Etikett angegeben in der Prov. Siirt,
sondern in der Prov. Hakkari.

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Electrophoretic differentiation and *Pieris napi* (L.)

(Lep. Pier.)

by

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A long standing controversy concerns the nature of the "species" and the point at which divergent populations may be termed separate species. One species group which has fueled this discussion is the *Pieris napi* group. Much argument has passed on the nature of different populations, with the Central European taxa being particularly well studied and disputed over (MÜLLER & KAUTZ, 1939; PETERSEN, 1952; BOWDEN, 1953, 1970; LORKOVIC, 1958, 1962; WARREN, 1961). The crucial issue is the status of the alpine forms *P. bryoniae* *P. bryoniae flavescens* etc. which have been variously regarded as separable from *P. napi* at the sub-specific, semi-specific or sibling species level. An accepted view among many Lepidopterists would now be that the speciation process appears far-advanced with some evidence for reproductive isolation of lowland and upland forms, at the western end of the Alpine chain, but possible hybridisation at the eastern end. The concept of the semi-species (LORKOVIC, 1962) seems useful here, where taxa are in the process of evolving reproductive isolation which is not yet complete. Recently this view has been challenged by

GEIGER (1978) who addressed the old problem with the new technique of electrophoretic detection of enzymatic allelic variants. GEIGER demonstrated genetic similarities at loci between *P. napi* and *P. bryoniae* and argued that the two were therefore conspecific. It is my purpose here to challenge both the results and interpretation of this study, and to argue that electrophoretic investigations need careful qualification in studies of speciation. During the period 1976–1980 I was engaged on an electrophoretic study of speciation in Pierinae (COURTNEY, 1980) which encompassed 22 loci in 23 taxa, with additional studies on local differentiation in 16 populations of *P. napi*, mostly within 40 km of northern England. It is not my purpose here to report the results of this extensive study (which compared to GEIGERs study of 10 loci in 4 taxa). It is sufficient here to use my results as a cautionary tale for all those who use electrophoresis to study speciation.

GEIGERs study must be qualified by it's lack of laboratory demonstrations of the genetic inheritance of his variants. Whilst these are often omitted in electrophoretic studies they can be important. In my own laboratory a PGM variant, apparently allelic, proved on close inspection to be a variably manifested larval enzyme, which produced a false "allele" in the bonding pattern. It cannot be asserted that GEIGERs study of PGM suffers from this inaccuracy since he used substantially different electrophoretic conditions. Nevertheless, the assumption of allelic basis for variants, particularly in monomeric enzymes, is demonstrably false and needs genetic studies for confirmation.

In my study of Pierinae I established that overall little allelic variation and genetic differentiation is present; taxa as diverse as *Pontia daplidice* and *Euchloe ausonia* having a value for NEIs Index of Identity (NEI, 1972) of $I = 0.454$ (Table 1). Studies on geographic populations and subspecies (*P. n. napi*, *P. n. bicolorata*; *P. rapae crucivora*, and *P. r. rapae* and *P. brassicae* both from populations in Britain, Malta and Morocco) indicate low differentiation at this level. Similarly low levels of divergence are found on comparing the semi-species pair (*P. (n.) bryoniae* and *P. n. napi*) and sibling-species pairs (*P. cheiranthi* and *P. brassicae*; *Euchloe ausonides* and *E. simplonia*; *P. napi* and *P. japonica*, *P. melete* and *P. virginensis*). Other high values of interest are those between *P. napi* and *P. rapae* ($I = 0.903$), *Anthocharis sara* and *A. belia* ($I = 0.939$), *E. simplonia* and *E. belemia* ($I = 0.940$), *E. belemia* and *Elphinstonia charlonia* ($I = 0.922$). The differences here between some of my values for I and those of GEIGER, which are typically much lower (e.g. *P. rapae* and *P. napi*, $I = 0.68$) are meaningless since our two studies are based on very different samples of loci. However, are we to agree with GEIGERs interpretation of high values for I ? He argues firstly that I for *P. n. napi* and *P. (n.) bryoniae* has a value near to 0.99 (my value is 0.9993); then since in studies of other insects, particularly *Drosophila* ssp., species pairs are typically much more highly differentiated (with I less than 0.8) *P. n. napi* and *P. (n.) bryoniae* cannot be separate species. This reasoning is fallacious on two counts. Firstly comparisons of values

of I from other species (Table 2) indicate that one cannot draw rules about the amount of genetic differentiation associated with speciation in all groups of organisms. In butterflies and mice, high values of I obtain between species, in *Drosophila* very low values. Of particular note is the fact that I for the comparison PAN v. HOMO is 0.54, equivalent to a sibling species on GEIGERs approach. I'm sure he would not wish to be considered a close relative of the chimpanzee! Secondly the assumption that two taxa are conspecific because they share a character, or not conspecific because of a lack is an old taxonomic error. BRITTNACHER et al. (1978) were unable to find any distinguishing electrophoretic characters between some very distinct *Speyeria* species. The only criterion for judging specific status is reproductive isolation. In this context GEIGERs results on gene frequencies are far more interesting and relevant than values for I .

One great advantage of GEIGERs studies over mine is that he used only field-collected samples and studied gene frequencies in populations. In those 16 populations where I studied local variation, it was clear that considerable differences in gene frequency at some loci (particularly Esterases) existed even between adjacent populations of lowland *P. n. napi*. Evidently studies of differentiation between taxa should therefore include several populations of each type. Within these limitations GEIGERs results do appear to show the important observation of similar gene frequencies in *P. n. napi* and *P. (n.) bryoniae*. What may be deduced from this?

Shared polymorphisms in two taxa may take three forms. Firstly, some variants may be shared but other alleles only found in one taxon; Secondly, the variants may all exist in the two taxa but at very different frequencies; Thirdly the gene frequencies may be similar. BRITTNACHER et al. (1978) give examples of all these *Speyeria* butterflies. The interpretation of such results is fraught with difficulties; in only the first case is the deduction clear — the two taxa do not share the same gene pool (there is reproductive isolation) and they must be regarded as good species. The second case may occur in closely related taxa due to the maintenance of ancestral characters (*P. napi* and *P. rapae* share such a polymorphism at a GOT locus) or may indicate a degree of introgression (semi-specific status) or convergent evolution (not unlikely with electrophoretic characters). The third case is the most interesting and problematical of all. Identical gene frequencies are indeed to be expected if there is little reproductive isolation (infra-specific status). However, comparisons at one or a few populations do not rule out either the possibility of shared ancestral gene frequencies or selective influences. If GEIGER had studied *P. (n.) bryoniae* and *P. n. napi* together at several localities and demonstrated between taxon differentiation was less than that observed between localities, this would be strong evidence for lack of reproductive isolation. Even here, to rule out the covarying effects of selection in similar species, several polymorphic loci would be necessary. However without this very detailed research, even gene frequency data can only

be suggestive as to species status.

In summary, GEIGER's study adds much useful information but no firm conclusion to the study of the status of *P. (n.) bryoniae*. Electrophoretic studies must be used very carefully when examining speciation. In this case there is one possible survey that would unequivocally settle the century-old controversy. A study is necessary in the western Alps of differentiation between the two taxa at, at least, five study sites where both occur in close proximity, and based on a minimum of four polymorphic loci. Covariance at all loci would argue very strongly for conspecificity. Lack of covariance would indicate a degree of reproductive isolation, or semi-specific status. Like most other Lepidopterists, I believe this latter to be the true state of affairs. No present electrophoretic survey, including my own, sufficiently addresses the nature of *P. bryoniae*, and electrophoretic studies cannot yet supplant earlier taxonomic work. It is to be hoped that here GEIGER will continue his research and attempt to clarify our understanding of this critical problem in speciation research.

Table 1: Mean values of NEIs index of Genetic Identity (I), based on electrophoretic comparisons of 23 taxa at 22 loci, for Pierinae at differing levels of evolutionary divergence. Sample sizes and standard errors are given.

Level of Divergence	n.	I	S.E.
Geographic populations			
Subspecies	8	0.997	0.001
Semi-species	1	0.9993	
Sibling species	8	0.958	0.011
Congeneric species	8	0.819	0.033
Sub Tribes	4	0.888	0.016
Contribal species	22	0.619	0.009
Tribes	32	0.403	0.007

Table 2: Mean values of I reported for taxa at progressive stages of divergence. 1. BRITTNACHER et al. (1978) 2. TURNER et al. (1979) 3. AYALA et al. (1974) 4. ZOUROUS (1973) 5. ZIMMERMAN et al. (1978)

Taxon	I subspecies	I semi-species	I sibling-species	I congeners
<i>Speyeria</i> butterflies 1	0.977			0.833
<i>Heliconius</i> butterflies ²	0.963			0.688
<i>Drosophila willistoni</i> 3	0.795	0.798	0.517	0.352
<i>Drosophila mulleri</i> 4	0.878		0.788	
<i>Peromyscus</i> deermice 5	0.949	0.837	0.945	0.716

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